Life Sciences 212 (2018) 30–36

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Systemic delivery of mesenchymal stem cells condition media in repeated doses acts as magic bullets in restoring IFN-γ/IL-4 balance in asthmatic rats

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ARTICLE INFO

Keywords:
Asthma
Conditioned media
T-bet
GATA-3

ABSTRACT

Aims: With respect to recent advantage in stem cell application, given the concern reported previously after stem cell transplantation, mesenchymal stem cell-derived conditioned media (MSC-CM) could be a potential approach to guarantee more safety and efficient outcomes than the current stem cell–based regenerative therapies.
Main methods: Male rats were assigned into four experimental groups (n = 6); healthy rats (C group), OVA sensitized rats (S group), OVA sensitized rats received a single dose of 50 μl CM intravenously (S group + SD-CM) and OVA sensitized rats received repeated doses of 50 μl CM intravenously (S group + RD-CM). Two weeks post-allergen challenge, the therapeutic effects of systemic administrated CM in single and repeated dosages were investigated by monitoring the transcription of T-bet, GATA-3, IL-4 and IFN-γ genes along with pathological changes in asthmatic lung tissue. IL-4 and IFN-γ levels and IFN-γ/IL-4 ratio were further evaluated in sera.
Key findings: Our data revealed that the systemic introduction of CM in repeated dosages could significantly reduce pathological injures in OVA-sensitized rats by the modulation of expression of T-bet and GATA-3 in lung tissues and interleukins levels ($p < 0.001$ to $p < 0.05$). In contrary, CM in single dosage did not yield any beneficial effect.
Significance: Overall, we indicated that systemic administration of CM in repeated dosages, but not in single dose, could be strategic approach in amelioration of asthmatic changes, presumably by the regulating the differentiation of naive CD4 T cells into Th1/Th2 effector cells via modulation of T-bet and GATA-3 expression in OVA-sensitized male rats.

1. Introduction

Asthma is a chronic lung disorder with profound socio-economic complications reaching 1 to 18% of the population in different countries while no definite therapeutic approaches have been defined yet [1]. Antigen-activated $CD4^+$ T-helper type-2 lymphocytes (Th2 cells) are the predominant T cell population in the allergic niche and play a key role in the initiation and regulation of the inflammatory responses in the asthmatic airways through the release of cytokines [2–4]. Chronic pathologic changes in the asthmatic airways are originally induced by Th2-driven inflammatory responses due to the shift in the Th1/Th2 balance in the favor Th2 polarization [3,5]. Therefore, novel and sophisticated therapeutic manipulations for asthma must focus on the restoration of Th1/Th2 balance as a key agent in the control of inflammatory rate in asthmatic airway [3,4,6]. Commensurate with these claims, the intrinsic immune-modulatory potential of mesenchymal stem cells (MSCs) is the driving force behind their application as a promising therapeutic paradigm for inflammatory diseases such as asthma [7]. MSCs are a population of multipotent adult stem cells that mainly found in bone marrow (BM-MSCs) niche [8]. They exert immune-modulatory activities by both differentiating into specific cell-types and secreting factors in paracrine behavior. Since, the survival and differentiation rate of stem cells in the inflammatory niche are significantly low, possibly due to ischemic microenvironment, it has been proposed that the paracrine-like fashion is principal mechanism for therapeutic effects of MSCs under the pathological condition [8–10].

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https://doi.org/10.1016/j.lfs.2018.09.049
Received 28 July 2018; Received in revised form 23 September 2018; Accepted 26 September 2018
Available online 27 September 2018

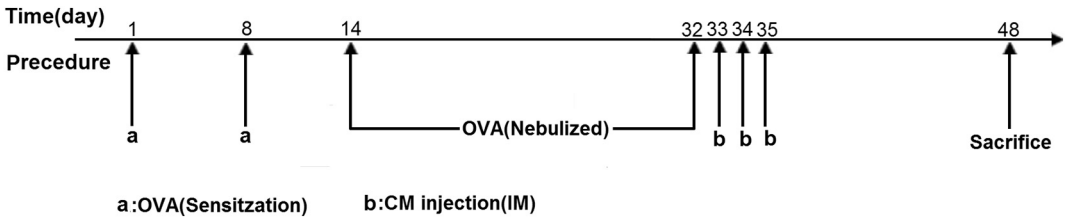

Fig. 1. Flow chart of experimental interventions.

It has been well-established that stem cell-secreted growth factors are responsible for some of their observed therapeutic effects, so it seems logical that the paracrine effects of MSCs could be monitored in their condition media (CM) [3]. Therefore, paracrine interaction hypothesis has inspired a novel alternative therapeutic modality that might warrant more efficient consequences than current stem cell-based therapies [11]. Increasing evidence in animal models of lung injury unveil that systemic administration of MSC-CM could protect the lung architecture against inflammation [10,12,15]. In contrary, we recently reported that systemic administration of MSC–CM in single dose has inert effects on the levels of Th1/Th2 cytokines in lung tissues of sensitized rats [3,7]. Authorities declared that therapeutic outcome solely depended on the CM volume and secretome profile [16–18]. Although the cost and benefits should be described for the continuous and repeated dose of CM injection, however, many reports unveiled the lack of rejection of CM in various experiments [16,17,19]. To our knowledge, there is a little data related to the repeated doses adminstration of MSC–CM in OVA-induced asthmatic rats. In line with this issue, more investigations are needed to unveil the therapeutic effect of CM administrated in repeated doses in comparison with single dose in asthmatic rats. Authors hypothesize that the results of the current experiment pave a way to highlight long-term and repeat doses of MSC secretome in modulating different inflammatory diseases.

2. Materials and methods

2.1. Ethics statement

All Animals were cared humanely according to the standards of the National Institutes of Health for Laboratory Animals Care and Use (NIH Publication No. 85-23, revised 1996) under the supervision of the Animal Research Ethics Board of Tabriz University of Medical Sciences (No: TBZMED.REC.1394.386).

2.2. Animal groups

Thirty healthy adult male Wistar rats (200–250 g, 6–8 weeks old) were enrolled to the current study. Six rats were randomly used for extraction of rat bone marrow-derived MSCs (rBMMSCs). The remaining 24 animals were assigned into four experimental groups (n = 6 per group) as follows:

1. Healthy rats (C group)
2. OVA-Sensitized rats (S group)
3. OVA-Sensitized rats received a single dose of 50 μl CM intravenously (S + SD-CM group)
4. OVA-Sensitized rats received repeated doses of 50 μl CM intravenously (S + RD-CM group)

2.3. Sensitization and challenge protocol

Rats in the sensitized groups were actively exposed to OVA over a period of 32 ± 1 days as described previously by our groups [3]. Briefly, on days 1 and 8, rats were sensitized by intra-peritoneal injection of a suspension containing 1 mg OVA (Sigma-Aldrich, USA) in 200 mg aluminum hydroxide as adjuvant solution. From days 14 to 32 ± 1, the sensitized rats were subjected to a single exposure of aerosolized 4% OVA by using an ultrasonic nebulizer (CX3, Omron Co., Netherland) coupled to a plastic inhalation chamber with dimensions 30 × 20 × 20 cm^3 for 5 min each day. In the control subjects, normal saline solution was applied instead of OVA in the same manner. After the completion of allergen challenge (day 33), the sensitized rats candidate to given CM were systemically injected via the femoral vein. In S + SD-CM group, rats were administrated by a single dose of 50 μl CM intravenously on day 33 while in S + RD-CM group, rats given repeated doses of 50 μl CM intravenously on three continuous days (days 33, 34 and 35). For the assessment of treatment efficacy, all rats were euthanized two weeks post-final allergen challenge (day 48) (Fig. 1).

2.4. MSC isolation and expansion

rBMMSCs were harvested from healthy rats as previously described [3,7]. In brief, bone marrow cells were evacuated from the femur medullary with pushing PBS containing 2% fetal bovine serum (FBS; Gibco, USA). The bone marrow mononuclear cell layer was then enriched by gradient centrifugation using Ficoll-hypaque® solution (Sigma, USA) at 400*g* for 20 min at room temperature. The mononuclear cells fraction was maintained in Dulbecco's modified Eagle's medium low glucose (DMEM/LG; Gibco, USA) with 20% FBS, 100 U/ml streptomycin and 100 mg/ml penicillin (Biosera, UK). Last, 1 × 10^5 cells were transferred into *6*-well culture plates (SPL). The exhausted medium was then replaced with fresh medium every 3–4 days to completely exclude non-adherent cells. For cell subculture, rBMMSCs were trypsinized by Trypsin-EDTA solution (Gibco, USA) after reaching 70–80% confluence and submitted for all the experimental purpose at passages three to six.

2.5. Enrichment of rat MSCs

The phenotype of cultured cells was confirmed by a panel of different monoclonal antibodies against cell surface markers, including PE-conjugated anti-CD45 as well as FITC-conjugated anti-CD34, CD44 and CD133 (all purchased from eBioscience, USA) [4]. Isotopes control antibodies were also used to measure the background fluorescence. Briefly, the cells were trypsinized by 0.025% Trypsin-EDTA solution (Gibco, *USA*) and blocked by 1% bovine serum albumin (Sigma, *USA*). Thereafter, an appropriate antibody concentration was added into 100 μl of PBS containing 5 × 10^5 cells and incubated away from light for 30 min. Ultimately, cells were introduced to BD FACSCalibur Flow Cytometer system (USA) and raw data were analyzed by Flow Jo software ver.7.6.1.

2.6. Conditioned media (CM) harvesting

To address the possible paracrine role of stem cells in alleviation of OVA-induced airway inflammation, CM of cultured MSCs was prepared [3,4]. Briefly, cells at 70–80% confluency were washed three times with PBS and incubated with FBS-free DMEM/LG. After 72 h, the supernatant was aspirated gently, centrifuged at 400*g* for 5 min, and filtered through 0.2-μm size syringe filter. CM was concentrated by using